Mem. Fac. Lit. & Sci., Shimane Univ., Nat. Sci., **1**, pp. 30–33, 2 text-figs., 1 table, March 15, 1968

Studies on the Blackish Substances found in *Eutrema Wasabi* afflicted with Black Leg Disease. II. Isolation of the Metabolic Products from the Culture of *Phoma Wasabiae*

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(Received November 30. 1967)

This investigation was undertaken to isolate the metabolic products from the blackish culture solution of potato produced by *Phoma Wasabiae*. It was carried out especially in order to examine the existence of carboxylic acids, phenols and coloring substances. In this report, some results from the preliminary experiments are described.

Experiments and Results

Medium and Culture Conditions. Culture medium was prepared as follows. In 1 l. of distilled water, 200 g. of the thinly sliced potato was boiled for 30 minutes, and the mixture was the filtered with 2 sheets of gauze. To the filtrate, 20 g. of glucose was added, the pH was adjusted to 7.0 with 20 % potassium hydroxide solution, and 400 ml. of the filtrate was poured into a 1-l. Erlenmeyer flask, then autoclaved at 120°C for 15 minutes.

Phoma Wasabiae was inoculated on the culture medium and incubated at 25°C for 3 or 4 weeks in the static state. After 3 or 4 weeks, the color of the culture medium turned blackish. The author calls this culture medium "blackish culture solution".

Isolation of the Metabolic Products. The blackish culture solution was treated according to a general method as shown in Fig. 1.

Divisions A, B, and C were extracted, respectively, with ether in acidity by

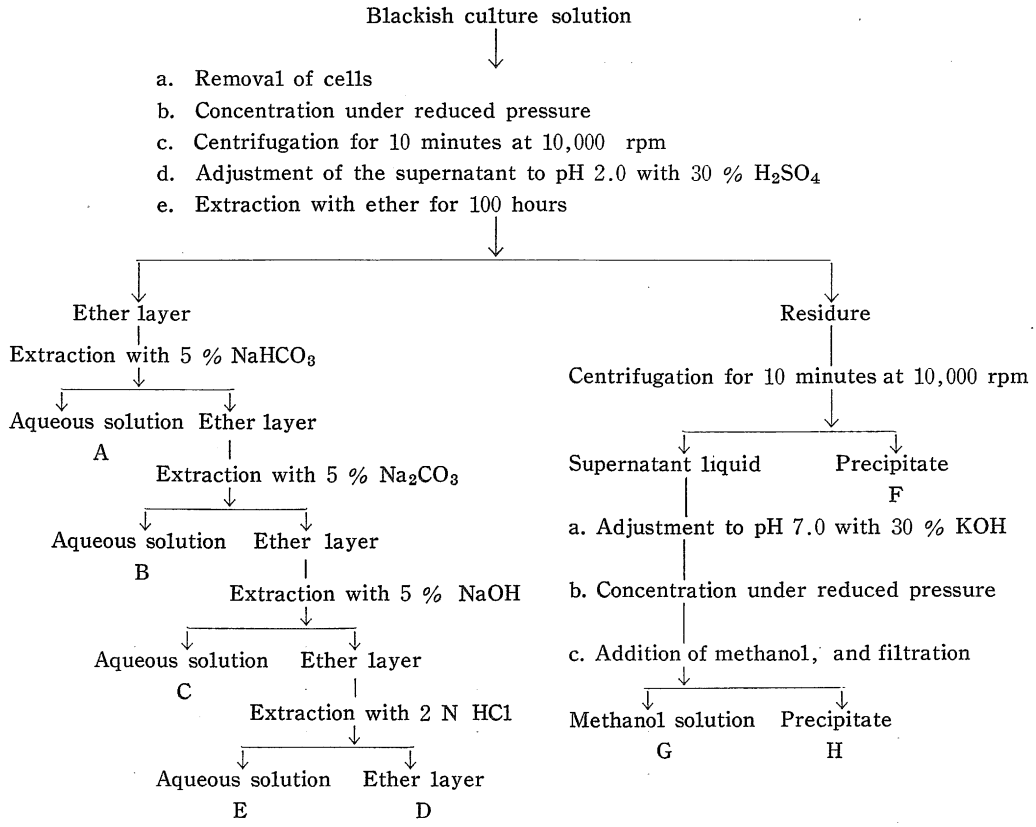

Fig. 1. Procedure of isolation of the metabolic products from the blackish culture solution produced by *Phoma Wasabiae*.

using 30 % H_2SO_4. The ether solution of division A had a deep reddish color, division B an orange-yellow color, divisions G and D idicated a pale-yellow color. From these ether solutions, ether was distilled off under reduced pressure. These concentrated extracts were kept in ampules, respectively. Precipitate F was a black substance. Methanol solution of division G had a dark reddish color, which resembled the color of soy. Precipitate H was dissolved in a small amount of water, and then methanol was added in such a way that methanol was in a final concentration of 80 %. When methanol added, a white-colored precipitate was produced, so that it was filtered again. These processes of extraction with methanol and the filtration were repeated until the color of methanol solution disappeared, which solution was added to division G and then distilled under reduced pressure.

Thin layer Chromatography. Divisions A, B, C, and D were used as samples.

Apparatus. Toyo Kagaku Sangyo Co., LTD, HC-type.

Preparation of Plates. Using Wakogel B-O, the thickness of a layer was

adjusted to 0.25 mm. The activated plates were made by heating at 105°C for 30 minutes and stored in a store box.

Development Procedure. A benzene-methanol mixture (95:5) was used as developing solvent. The ascending method was applied ; the development chamber was saturated with the developing solvent, and then by the use of capillary tube, a proper quantity of sample was spotted 1.5 cm. from the bottom of a plate. The plate was then placed in the development chamber and the solvent was allowed to travel 10 cm. from the origin. The developed chromatogram was observed under a visible light and 365.0-mμ light source, then sprayed with the following color reagents.

(1) Bromocresol green (indicator), (2) Tollen's reagent, (3) ferricchloride, (4) Pauly's reagent, (5) 2, 4-dinitrophenylhydrazine. As the results of the experiments, the following phenomena were observed. (Fig. 2, Table 1.)

Table 1. Reactions with the color reagents

Reagents Spot Rf*	Indicator (Bromocresol green)	Tollen's reagent	Ferric chloride	Pauly's reagent	2,4-Dinitrophenylhydrazine
A.1 0.83	+ yellow	+ black	−	+ orange	+ orange
B.1 0.80	+ yellow	+ black	+ red violet	+ orange	+ orange
C.1 0.75	+ yellow	+ black	−	+ yellow	+ orange
C.2 0.42	?	+ black	+ red violet	+ orange	+ orange red
D.1 0.87	?	+ black	?	?	?

\+ : positive

− : negative

* As the developing solvent shows a marginal phenomenon, all the Rf values of the spots are not constant.

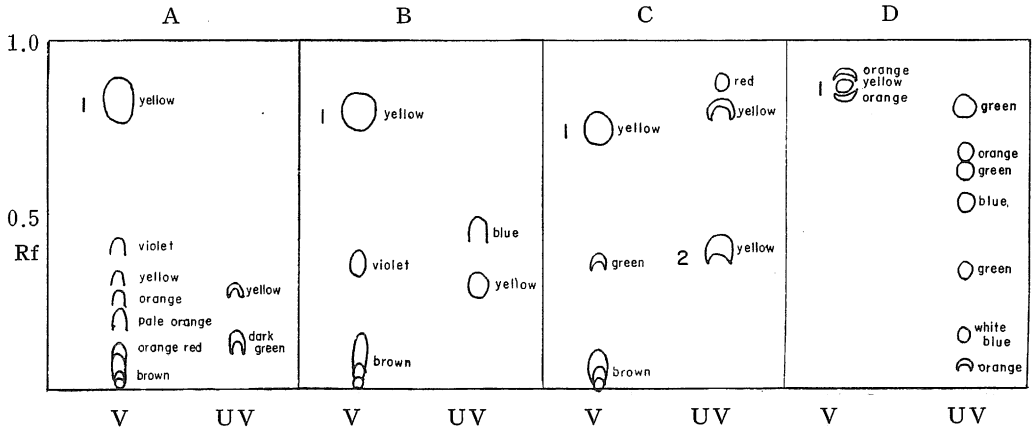

Fig. 2. Thin layer chromatograms of divisions A, B, C, and D.

V : Under visible light

UV : Under ultraviolet ray

1) Under a visible light, coloring substances were most numerous in division A, but fluorescent substances were numerous in division D.

2) Some yellowish substances were recognized on the chromatogram of each division.

3) The spot of B. 1 turned black on contact with air.

4) The spot of C. 2 showed a yellowish fluorescence, and reacted with the color reagents.

5) Division D did not react so easily with these reagents.

Comparison with Caffeic Acid. Yellowish caffeic acid, which is a kind of polyhydric phenol, has been found in the black-rotted sweet potato[1], or in the potato afflicted with various pathogenic organisms.[2),3)] These yellowish substances were, therefore, compared with caffeic acid. As the results of the experiments, the following facts were found.

i) Caffeic acid could not be developed in the same solvent (benzene : methanol=95 : 5).

ii) It showed a green color with ferric chloride solution.

iii) It showed a pale-brown color with the Pauly's reagent.

Thus it was ascertained that none of these yellowish substances is identical with caffeic acid.

Summary

The investigation was undertaken in order to isolate the metabolic products of *Phoma Wasabiae*, incubated for 3 or 4 weeks at 25°C on the liquid culture medium of patato.

Acidic, phenolic, and coloring substances were isolated from the blackish culture solution.

The results of developing by the use of thin layer chromatography, showed that the yellowish substances are not caffeic acid.

The author expresses his deep gratitude to Professor Ryozo GOTO of Kyoto University for many helpful discussions and suggestions during this work.

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